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# A new species of the *Boulenophrys* (Anura, Megophryidae) from Hubei, China

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## Abstract

A new species of Asian horned toad, *Boulenophrys*, is described from Changyang County, Hubei Province, China. The new species differs from its congeners by the following combination of characters: (1) body size moderate (SVL 39.4–43.3 mm in males); (2) a small horn-like tubercle at the edge of each upper eyelid; (3) vomerine ridges weak and vomerine teeth absent; (4) tongue not notched posteriorly; (5) head width greater than head length; (6) no large, pale-white circular spots on the shoulders; (7) toes without webbing or lateral fringes; (8) heels overlapping when thighs are positioned at right angles to the body; (9) tibiotarsal articulation reaching the level of the middle of the eye when the leg is stretched forward; (10) an internal single subgular vocal sac in males; and (11) in breeding males, nuptial pads bearing black nuptial spines on the dorsal bases of the first and second fingers.

## Key Words

Hubei Province, Molecular phylogenetic analysis, Morphology, New species

## Introduction

The subfamily Megophryinae (Bonaparte, 1850) is widely distributed in eastern and central China, throughout southeastern Asia, and extends to the islands of the Sunda Shelf and the Philippines, westward to Nepal and northeastern India (Frost 2025). The taxonomic arrangements, especially regarding the generic assignments within the group, have long been controversial (e.g., Tian and Hu 1983; Dubois 1987; Lathrop 1997; Rao and Yang 1997; Delorme et al. 2006; Jiang et al. 2008; Fei et al. 2009; Fei and Ye 2016; Chen et al. 2017; Mahony et al. 2017; Qi et al. 2021; Lyu et al. 2023). Recently, Lyu et al. (2023) reviewed the literature comprehensively and conducted morphological and molecular phylogenetic analyses, proposing a revised generic classification for Megophryinae. Among these genera, *Boulenophrys* is one of the most widely distributed and speciose, comprising 71 reported species. The genus has been documented across most of China and extends south into Vietnam, Laos, Thailand, and Myanmar; with less certainty, it also occurs in Bhutan and northeastern India. Notably, 47 species of this genus were described in the past decade (Frost 2025). Clearly, the diversity of *Boulenophrys* has been underestimated, and more undescribed species likely remain to be discovered.

During a recent amphibian survey in Changyang County, Hubei Province, China, five specimens of Asian horned toads were collected. Molecular phylogenetic

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analyses and morphological comparisons indicate that these specimens represent a new taxon. Herein, we describe it as a new species.

# Materials and methods

## Sampling

Five adult males of the undescribed species were collected in Changyang County, Hubei Province, China (Fig. 1). In the field, the toads were euthanized using isoflurane, and the specimens were fixed in 75% ethanol. Tissue samples were taken and preserved separately in 95% ethanol prior to fixation. The specimens were deposited in Moutai Institute (**MT**).

## Molecular data and phylogenetic analyses

DNA was extracted from muscle tissue using a DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing). Two fragments of the mitochondrial 16S rRNA (16S) and cytochrome oxidase subunit I (COI) genes were amplified. For 16S, primers P7 (5′-CGCCTGTTTACCAAAAACAT-3′) and P8 (5′-CCGGTCTGAACTCAGATCACGT-3′) were used (Simon et al. 1994); for COI, Chmf4 (5′-TYTCWACWAAYCAYAAAGAYATCGG-3′) and Chmr4 (5′-ACYTCRGGRTGRCCRAARAATCA-3′) were used (Che et al. 2012). Gene fragments were amplified under the following conditions: an initial denaturing step at 95 °C for 4 min; 36 cycles of denaturing at 95 °C for 30 s, annealing at 52 °C (for 16S) or 47 °C (for COI) for 40 s, and extension at 72 °C for 70 s. Sequencing was conducted using an ABI 3730 automated DNA sequencer (Shanghai DNA BioTechnologies Co., Ltd., Shanghai, China). New sequences were deposited in GenBank (for GenBank accession numbers, see Table 1).

For phylogenetic and genetic divergence analyses, we downloaded available corresponding sequence data for all related species from GenBank based on previous studies (Liu et al. 2018; Song et al. 2024; Wang et al. 2024; Table 1). Sequences were assembled and aligned using the ClustalW module in BioEdit v. 7.0.9.0 (Hall 1999) with default settings. Based on the concatenated 16S + COI dataset, phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) methods, implemented in PhyML 3.0 (Guindon et al. 2010) and MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003), respectively. The best-fit model (GTR + G + I) was selected using the Bayesian information criterion (BIC) in PartitionFinder 2 (Lanfear et al. 2012).

For ML analysis in PhyML 3.0, the bootstrap consensus tree was inferred from 1000 replicates to estimate nodal support. For BI analysis in MrBayes v. 3.1.2, four Markov chains were run for 50 million generations with sampling every 1000 generations, until convergence (split frequency < 0.05) was reached. The first 25% of trees were discarded as burn-in, followed by calculation of Bayesian posterior probabilities and construction of a 50% majority-rule consensus tree

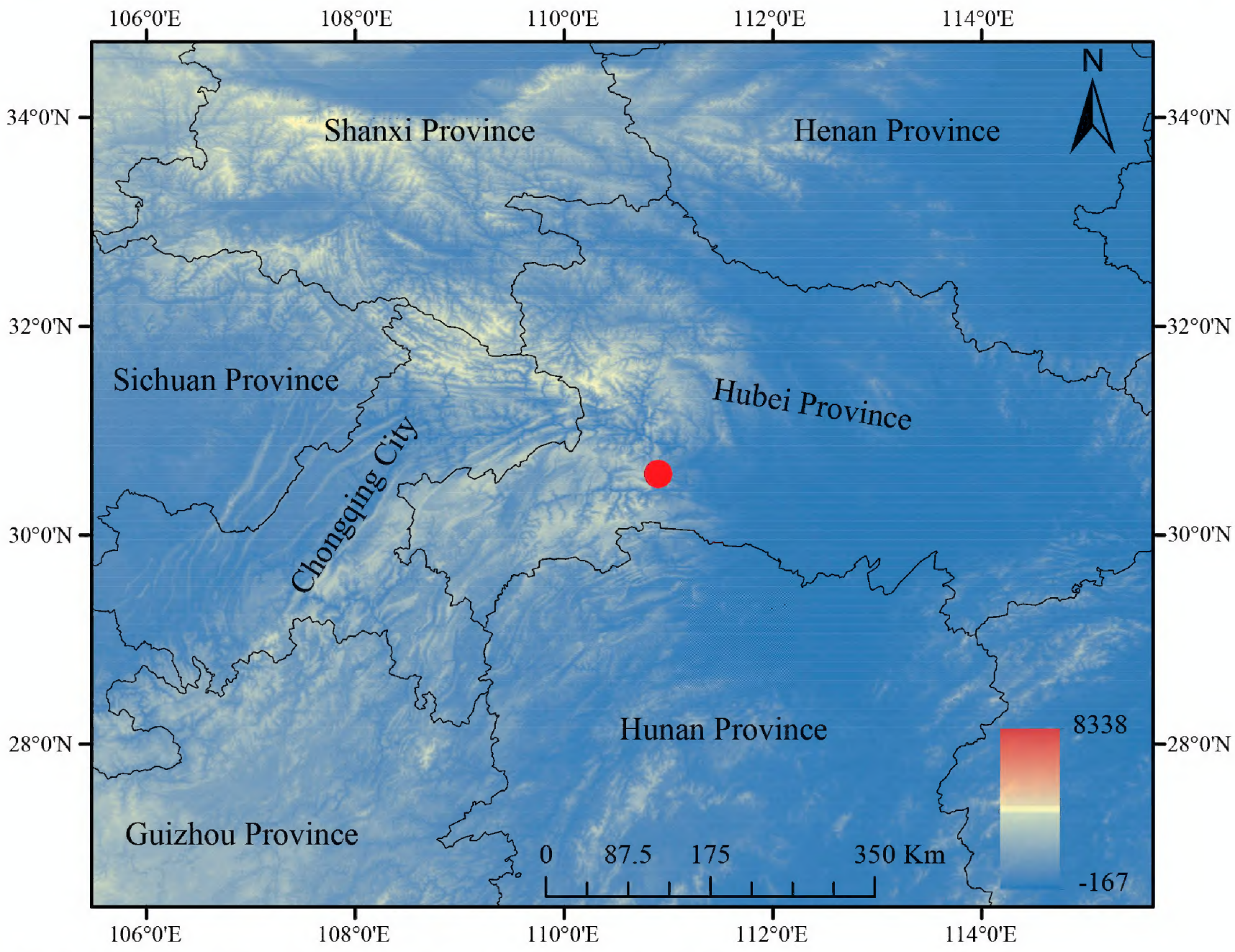


**Figure 1.** Map showing the distribution sites of *Boulenophrys changyangensis* sp. nov.

**Table 1.** Information for samples used in molecular phylogenetic analyses in this study.

| ID | Species | Voucher | Locality | GenBank accession number | |
|---|---|---|---|---|---|
| | | | | 16S | COI |
| 1 | *B. changyangensis* sp. nov. | MT CY20220806005 | China, Hubei, Changyang | PV768530 | PV768629 |
| 2 | *B. changyangensis* sp. nov. | MT CY20220806004 | China, Hubei, Changyang | PV768529 | PV768628 |
| 3 | *B. changyangensis* sp. nov. | MT CY20220806006 | China, Hubei, Changyang | PV768531 | PV768630 |
| 4 | *B. changyangensis* sp. nov. | MT CY20220806002 | China, Hubei, Changyang | PV768527 | PV768626 |
| 5 | *B. changyangensis* sp. nov. | MT CY20220806003 | China, Hubei, Changyang | PV768528 | PV768627 |
| 6 | *B. yingdeensis* | SYS a004721 | China, Guangdong, Yingde | MH406846 | MH406308 |
| 7 | *B. xiangnanensis* | SYS a002874 | China, Hunan, Shuangpai | MH406713 | MH406165 |
| 8 | *B. nanlingensis* | SYS a001962 | China, Guangdong, Ruyuan | MH406645 | MH406081 |
| 9 | *B. lini* | KIZ 07053 | China, Jiangxi, Mt Jinggang | KX811842 | KX812110 |
| 10 | *B. kuatunensis* | SYS a003449 | China, Jiangxi, Mt Wuyi | MF667881 | MH406206 |
| 11 | *B. daiyunensis* | SYS a007711 | China, Fujian, Tong'an | MW367054 | MW365504 |
| 12 | *B. puningensis* | SYS a005770 | China, Guangdong, Puning | OL635585 | OL634853 |
| 13 | *B. fengshunensis* | SYS a004724 | China, Guangdong, Fengshun | MH406848 | MH406310 |
| 14 | *B. sanmingensis* | SYS a007057 | China, Jiangxi, Mt Magu | MW367051 | MW365501 |
| 15 | *B. baishanzuensis* | CIB QY20200719002 | China, Zhejiang, Qingyuan | MW001151 | MT998292 |
| 16 | *B. pepe* | GEP a207 | China, Guangdong, Qingyuan | PQ131151 | PQ130479 |
| 17 | *B. obesa* | SYS a002275 | China, Guangdong, Fengkai | KJ579123 | MH406125 |
| 18 | *B. ombrophila* | CIB WY18082308 | China, Fujian, Mt Wuyi | MW001159 | MT998300 |
| 19 | *B. cheni* | SYS a004050 | China, Jiangxi, Mt Jinggang | MF667873 | MH406241 |
| 20 | *B. nankunensis* | SYS a004503 | China, Guangdong, Mt Nankun | MH406824 | MH406286 |
| 21 | *B. dongguanensis* | SYS a002007 | China, Guangdong, Dongguan | MH406654 | MH406090 |
| 22 | *B. wugongensis* | SYS a004801 | China, Jiangxi, Anfu | MH406854 | MH406316 |
| 23 | *B. insularis* | SYS a002171 | China, Guangdong, Nan'ao | MH406665 | MH406105 |
| 24 | *B. hungtai* | SYS a007577 | China, Guangdong, Jiexi | OL635594 | OL634861 |
| 25 | *B. elongata* | GEPa155 | China, Guangdong, Huizhou | OR601595 | OR597101 |
| 26 | *B. lichun* | CIB 121428 | China, Fujian, Ningde | PQ309137 | PQ300665 |
| 27 | *B. brachykolos* | SYS a002258 | China, Hong Kong | KJ560403 | MH406120 |
| 28 | *B. xianjuensis* | CIB XJ190503 | China, Zhejiang, Xianju | MN563758 | MN563774 |
| 29 | *B. daoji* | SYS a006212 | China, Zhejiang, Mt Tiantai | MW367047 | MW365497 |
| 30 | *B. lishuiensis* | SYS a008445 | China, Zhejiang, Liandu | OQ180984 | OQ180872 |
| 31 | *B. tongboensis* | SYS a003227 | China, Jiangxi, Mt Tongbo | MH406744 | MH406201 |
| 32 | *B. jinggangensis* | SYS a004028 | China, Jiangxi, Mt Jinggang | MH406780 | MH406239 |
| 33 | *B. baolongensis* | KIZ 019216 | China, Chongqing, Wushan | KX811813 | KX812093 |
| 34 | *B. wushanensis* | KIZ YPX47799 | China, Chongqing, Wushan | KX811835 | / |
| 35 | *B. tuberogranulata* | KIZ YPX10987 | China, Hunan, Sangzhi | KX811823 | KX812095 |
| 36 | *B. gaolanensis* | SYS a009225 | China: Guangdong: Zhuhai | PQ217847 | PQ218778 |
| 37 | *B. acuta* | SYS a002266 | China, Guangdong, Fengkai | KJ579119 | MH406122 |
| 38 | *B. yangmingensis* | SYS a002888 | China, Hunan, Shuangpai | MH406719 | MH406171 |
| 39 | *B. shimentaina* | SYS a002077 | China, Guangdong, Yingde | MH406655 | MH406092 |
| 40 | *B. jiulianensis* | SYS a004218 | China, Jiangxi, Mt Jiulian | MH406790 | MH406252 |
| 41 | *B. yunkaiensis* | SYS a004694 | China, Guangdong, Xinyi | MH406845 | MH406307 |
| 42 | *B. yaoshanensis* | SYS a004878 | China, Guangxi, Jinxiu | MH406863 | MH406325 |
| 43 | *B. leishanensis* | KIZ 049172 | China, Guizhou, Mt Leigong | KX811825 | KX812102 |
| 44 | *B. congjiangensis* | GZNU 20200706003 | China, Guizhou, Congjiang | MW959773 | MW959761 |
| 45 | *B. shunhuangensis* | HNNU 16SH04 | China, Hunan, Mt Shunhuang | MK836027 | / |
| 46 | *B. mirabilis* | SYS a002289 | China, Guangxi, Lingui | MH406681 | MH406127 |
| 47 | *B. xuefengmontis* | SYS a007227 | China, Hunan, Mt Xuefeng | OQ180973 | OQ180861 |
| 48 | *B. hengshanensis* | CSUFT HS210612 | China, Hunan, Mt Hengshan | ON209291 | / |
| 49 | *B. liboensis* | GNUG 20150813001 | China, Guizhou, Libo | MF285253 | / |
| 50 | *B. boettgeri* | KIZ YPXJK033 | China, Fujian, Mt Wuyi | KX811814 | KX812104 |
| 51 | *B. mufumontana* | SYS a006419 | China, Hunan, Mt Mufu | MK524107 | MK524138 |
| 52 | *B. caudoprocta* | SYS a004293 | China, Hunan, Sangzhi | MH406796 | MH406258 |
| 53 | *B. sangzhiensis* | SYS a004313 | China, Hunan, Sangzhi | MH406802 | MH406264 |
| 54 | *B. fanjingmontis* | SYS a004350 | China, Guizhou, Mt Fanjing | MH406808 | MH406270 |
| 55 | *B. spinata* | CIBLS20190801002 | China,Guizhou,Mt Leigong | MT651551 | MT654518 |
| 56 | *B. qianbeiensis* | CIBTZ20190608015 | China,Guizhou,Tongzi | MT651553 | MT654520 |
| 57 | *B. binlingensis* | SYSa005314 | Wawu Shan, Sichuan, China | MH406893 | MH406355 |
| 58 | *B. binchuanensis* | KIZ 019441 | China, Yunnan, Mt Jizu | KX811849 | KX812112 |
| 59 | *B. omeimontis* | KIZ 025765 | China, Sichuan, Mt Emei | KX811884 | KX812136 |
| 60 | *B. daweimontis* | KIZ 048997 | China, Yunnan, Mt Dawei | KX811867 | KX812125 |
| 61 | *B. anlongensis* | CIB AL20190531018 | China, Guizhou, Anlong | MT823184 | MT823261 |
| 62 | *B. wuliangshanensis* | SYS a003924 | China, Yunnan, Mt Wuliang | MH406771 | MH406230 |

| ID | Species | Voucher | Locality | GenBank accession number | |
|---|---|---|---|---|---|
| | | | | 16S | COI |
| 63 | *B. jingdongensis* | SYS a003928 | China, Yunnan, Mt Wuliang | MH406773 | MH406232 |
| 64 | *B. caobangensis* | IEBR 4385 | Vietnam, Cao Bang, Nguyen Binh | LC483945 | / |
| 65 | *B. rubrimera* | AMS R177676 | Vietnam, Lao Cai, Sapa | MF536419 | MW086542 |
| 66 | *B. palpebralespinosa* | KIZ 011650 | Vietnam, Thanh Hoa, Pu Hu | KX811889 | KX812138 |
| 67 | *B. angka* | KIZ 040591 | Thailand, Chiang Mai | MN508052 | / |
| 68 | *B. lushuiensis* | CIB YN201909289 | China, Yunnan, Lushui | MW001226 | MW000913 |
| 69 | *B. minor* | KIZ YPX37545 | China, Sichuan, Dujiangyan | KX811895 | KX812144 |
| 70 | *B. jiangi* | CIB KKS20180722006 | China, Guizhou, Kuankuoshui | MN107743 | MN107748 |
| 71 | *B. chishuiensis* | SYS a004953 | China, Guizhou, Chishui | MH406867 | MH406329 |
| 72 | *B. hoanglienensis* | VNMN 2018.02 | Vietnam, Lao Cai, Sapa | MH514889 | MW086551 |
| 73 | *B. frigida* | AMS R186131 | Vietnam, Lao Cai, Bat Xat | MT364279 | MW086550 |
| 74 | *B. fansipanensis* | AMS R186115 | Vietnam, Lao Cai, Sapa | MH514887 | MW086548 |
| 75 | *Xenophrys glandulosa* | SYS a003757 | China, Yunnan, Mt Gaoligong | MH406754 | MH406213 |
| 76 | *Xenophrys mangshanensis* | SYS a002177 | China, Guangdong, Huaiji | MH406666 | MH406106 |

based on the post-burn-in samples. Finally, uncorrected p-distances (1000 replicates) between species based on 16S and COI were calculated in MEGA v. 6.06 (Tamura et al. 2013).

## Morphological comparisons

Morphological measurements were made with dial calipers to the nearest 0.1 mm. Eighteen morphometric characters of five adult specimens were measured following Fei et al. (2009):

**ED** eye diameter (distance from the anterior corner to the posterior corner of the eye);
**FL** foot length (distance from tarsus to the tip of the fourth toe);
**HDL** head length (distance from the tip of the snout to the articulation of the jaw);
**HDW** maximum head width (greatest width between the left and right articulations of the jaw);
**HLL** hindlimb length (maximum length from the vent to the distal tip of the Toe IV);
**IND** internasal distance (minimum distance between the inner margins of the external nares);
**IOD** interorbital distance (minimum distance between the inner edges of the upper eyelids);
**LAL** length of lower arm and hand (distance from the elbow to the distal end of the Finger IV);
**LW** lower arm width (maximum width of the lower arm);
**ML** manus length (distance from tip of third digit to proximal edge of inner palmar tubercle);
**SVL** snout-vent length (distance from the tip of the snout to the posterior edge of the vent);
**SL** snout length (distance from the tip of the snout to the anterior corner of the eye);
**TFL** length of foot and tarsus (distance from the tibiotarsal articulation to the distal end of the Toe IV);
**THL** thigh length (distance from vent to knee),
**TL** tibia length (distance from knee to tarsus);
**TW** maximal tibia width;
**TYD** maximal tympanum diameter;
**UEW** upper eyelid width (greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis).

Sex determination was based on the direct observation of calls or the presence of internal vocal sac openings in males. The presence or absence of nuptial pads was examined under a dissecting microscope. The new species was also compared morphologically with all other *Boulenophrys* species. Comparative data were obtained from related species as described in the literature (Table 2).

# Results

## Phylogenetic analyses

A total of 539 base pairs (bp) of the 16S gene and 555 bp of the COI gene were concatenated. ML and BI trees based on the mitochondrial DNA dataset showed almost consistent topologies, although relationships among many lineages remained unresolved (Fig. 2), with strong support for the major nodes in both analyses. In both ML and BI trees, all samples of the genus *Boulenophrys* from this study clustered within a single group, forming a distinct lineage with high nodal support (BPP = 1.00; BS = 100%). The topology also suggested a potential sister relationship between this clade and *B. baolongensis*, though this relationship received low support (BPP < 0.60; BS < 50%) (Fig. 2).

Additionally, the mean genetic distance between the undescribed taxon and its closely related species *B. baolongensis* was 2.3% for 16S and 9.0% for COI. The genetic distance from the new taxon to *B. wushanensis* was 2.9% for 16S, and to *B. tuberogranulata* was 1.9% for 16S and 6.7% for COI. These values exceed interspecific thresholds observed in many congeneric species pairs within *Boulenophrys* (Suppl. materials S1, S2). Furthermore, the specimens are distinguishable from other congeners in the genus by a unique combination of morphological characters (see taxonomic account below).

## Morphological analyses

Morphological comparisons for the undescribed taxon with other recognized species of the *Boulenophrys* genus are listed in Suppl. material 3.

**Table 2.** References for morphological characters for congeners of the genus *Boulenophrys*.

| ID | Species | References |
|---|---|---|
| 1 | *B. acuta* Wang, Li & Jin, 2014 | Li et al. 2014 |
| 2 | *B. angka* Wu, Suwannapoom, Poyarkov, Pawangkhanant, Xu, Jin, Murphy & Che, 2019 | Wu et al. 2019 |
| 3 | *B. anlongensis*Li, Lu, Liu & Wang, 2020 | Li et al. 2020 |
| 4 | *B. baishanzuensis* Wu, Li, Liu, Wang & Wu, 2020 | Wu et al. 2020 |
| 5 | *B. baolongensis* Ye, Fei & Xie, 2007 | Ye et al. 2007 |
| 6 | *B. binchuanensis* Ye & Fei, 1995 | Ye and Fei 1995; Lyu et al. 2023 |
| 7 | *B. binlingensis* Jiang, Fei & Ye, 2009 | Fei et al. 2009; Lyu et al. 2023 |
| 8 | *B. boettgeri* Boulenger, 1899 | Boulenger 1899; Lyu et al. 2023 |
| 9 | *B. brachykolos* Inger & Romer, 1961 | Inger and Romer 1961; Lyu et al. 2023 |
| 10 | *B. caobangensis* Nguyen, Pham, Nguyen, Luong & Ziegler, 2020 | Nguyen et al. 2020 |
| 11 | *B. caudoprocta* Shen, 1994 | Shen. 1994 |
| 12 | *B. cheni* Wang & Liu, 2014 | Wang et al. 2014 |
| 13 | *B. chishuiensis* Xu, Li, Liu, Wei & Wang, 2020 | Xu et al. 2020 |
| 14 | *B. congjiangensis* Luo, Wang, Wang, Lu, Wang, Deng & Zhou, 2021 | Luo et al. 2021 |
| 15 | *B. daiyunensis* Lyu, Wang & Wang, 2021 | Lyu et al. 2021 |
| 16 | *B. daoji* Lyu, Zeng, Wang & Wang, 2021 | Lyu et al. 2021 |
| 17 | *B. daweimontis* Rao & Yang, 1997 | Rao and Yang 1997 |
| 18 | *B. dongguanensis* Wang & Wang, 2019 | Wang et al. 2019b |
| 19 | *B. dupanglingensis* Xiao & Mo, 2025 | Xiao et al. 2025 |
| 20 | *B. elongata* Zeng, Wang, Chen, Xiao, Zhan, Li & Lin, 2024 | Zeng et al. 2024 |
| 21 | *B. fanjingmontis* Zhang, Liang, Ran & Shen, 2012 | Zhang et al. 2012 |
| 22 | *B. fansipanensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen,Nguyen, Portway, Luong & Rowley, 2018 | Tapley et al. 2018 |
| 23 | *B. fengshunensi* Wang, Zeng, Lyu & Wang, 2022 | Wang et al. 2022 |
| 24 | *B. frigida* Tapley, Cutaja, Nguyen, Portway, Mahony, Nguyen, Harding, Luong& Rowley, 2021 | Tapley et al. 2021 |
| 25 | *B. gaolanensis* Song, Wang,Qi, Wang, Wang,2024 | Song et al.2024 |
| 26 | *B. hengshanensis* Qian, Hu, Mo, Gao, Zhang & Yang, 2023 | Qian et al. 2023 |
| 27 | *B. hoanglienensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen,Nguyen, Portway, Luong & Rowley, 2018 | Tapley et al. 2018 |
| 28 | *B. hungtai Wang, Zeng, Lyu, Xiao & Wang, 2022* | Wang et al. 2022 |
| 29 | *B. insularis* Wang, Liu, Lyu, Zeng & Wang, 2017 | Wang et al. 2017a |
| 30 | *B. jiangi* Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020 | Liu et al. 2020 |
| 31 | *B. jingdongensis* Fei & Ye, 1983 | Fei et al. 1983; Lyu et al. 2023 |
| 32 | *B. jinggangensis* Wang, 2012 | Wang et al. 2012 |
| 33 | *B. jiulianensis* Wang, Zeng, Lyu & Wang, 2019 | Wang et al. 2019b |
| 34 | *B. kuatunensis* Pope, 1929 | Pope 1929; Lyu et al. 2023 |
| 35 | *B. leishanensis* Li, Xu, Liu, Jiang, Wei & Wang, 2018 | Li et al. 2018 |
| 36 | *B. liboensis* Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017 | Zhang et al. 2017 |
| 37 | *B. lichun* Lin, Chen, Li, Peng, Zeng, Wang, 2024 | Lin et al.2024 |
| 38 | *B. lini* Wang & Yang, 2014 | Wang et al. 2014 |
| 39 | *B. lishuiensis* Wang, Liu & Jiang, 2017 | Wang et al. 2017b |
| 40 | *B. lushuiensis* Shi, Li, Zhu, Jiang, Jiang & Wang, 2021 | Shi et al. 2021 |
| 41 | *B. minor* Stejneger, 1926 | Stejneger 1926; Lyu et al. 2023 |
| 42 | *B. mirabilis* Lyu, Wang & Zhao, 2020 | Lyu et al. 2020 |
| 43 | *B. mufumontana* Wang, Lyu & Wang, 2019 | Wang et al. 2019b |
| 44 | *B. nankunensis* Wang, Zeng & Wang, 2019 | Wang et al. 2019b |
| 45 | *B. nanlingensis* Lyu, Wang, Liu & Wang, 2019 | Wang et al. 2019b |
| 46 | *B. obesa* Wang, Li & Zhao, 2014 | Li et al. 2014 |
| 47 | *B. ombrophila* Messenger & Dahn, 2019 | Messenger et al. 2019 |
| 48 | *B. omeimontis* Liu, 1950 | Liu 1950; Lyu et al. 2023 |
| 49 | *B. palpebralespinosa* Bourret, 1937 | Bourret 1937; Lyu et al. 2023 |
| 50 | *B. pepe* Wang & Zeng, 2024 | Wang et al. 2024 |
| 51 | *B. puningensis* Wang, Zeng, Lyu, Xiao & Wang, 2022 | Wang et al. 2022 |
| 52 | *B. qianbeinsis* Su, Shi, Wu, Li, Yao, Wang & Li, 2020 | Su et al. 2020 |
| 53 | *B. rubrimera* Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley,2017 | Tapley et al. 2017 |
| 54 | *B. sangzhiensis* Jiang, Ye & Fei, 2008 | Jiang et al. 2008 |
| 55 | *B. sanmingensis* Lyu & Wang, 2021 | Lyu et al. 2021 |
| 56 | *B. shimentaina* Lyu, Liu & Wang, 2020 | Lyu et al. 2020 |
| 57 | *B. shuichengensis* Tian & Sun, 1995 | Tian and Sun 1995 |
| 58 | *B. shunhuangensis* Wang, Deng, Liu, Wu & Liu, 2019 | Wang et al. 2019a |
| 59 | *B. spinata* Liu & Hu, 1973 | Hu et al. 1973; Lyu et al. 2023 |
| 60 | *B. tongboensis* Wang & Lyu, 2021 | Lyu et al. 2021 |
| 61 | *B. tuberogranulatus* Shen, Mo & Li, 2010 | Mo et al. 2010 |
| 62 | *B. wugongensis* Wang, Lyu & Wang, 2019 | Wang et al. 2019b |
| 63 | *B. wuliangshanensis* Ye & Fei, 1995 | Ye and Fei 1995 |
| 64 | *B. wushanensis* Ye & Fei, 1995 | Ye and Fei 1995 |

| ID | Species | References |
|---|---|---|
| 65 | *B. xiangnanensis* Lyu, Zeng & Wang, 2020 | Lyu et al. 2020 |
| 66 | *B. xianjuensis* Wang, Wu, Peng, Shi, Lu & Wu, 2020 | Wang et al. 2020 |
| 67 | *B. xuefengmontis* Lyu & Wang, 2023 | Lyu et al. 2023 |
| 68 | *B. yangmingensis* Lyu, Zeng & Wang, 2020 | Lyu et al. 2020 |
| 69 | *B. yaoshanensis* Qi, Mo, Lyu, Wang & Wang, 2021 | Qi et al. 2021 |
| 70 | *B. yingdeensis* Qi, Lyu, Wang & Wang, 2021 | Qi et al. 2021 |
| 71 | *B. yunkaiensis* Qi, Wang, Lyu & Wang, 2021 | Qi et al. 2021 |

By having a medium body size, The undescribed taxon distinguished from *B. acuta*, *B. angka*, *B. baishanzuensis*, *B. binchuanensis*, *B. boettgeri*, *B. brachykolos*, *B. caobangensis*, *B. cheni*, *B. congjiangensis*, *B. daiyunensis*, *B. daoji*, *B. daweimontis*, *B. elongata*, *B. frigida*, *B. gaolanensis*, *B. hungtai*, *B. jinggangensis*, *B. jiulianensis*, *B. kuatunensis*, *B. leishanensis*, *B. lichun*, *B. lishuiensis*, *B. lushuiensis*, *B. mufumontana*, *B. nankunensis*, *B. nanlingensis*, *B. obesa*, *B. ombrophila*, *B. palpebralespinosa*, *B. pepe*, *B. puningensis*, *B. rubrimera*, *B. sanmingensis*, *B. shimentaina*, *B. shunhuangensis*, *B. tongboensis*, *B. wugongensis*, *B. wuliangshanensis*, *B. wushanensis*, *B. xianjuensis*, *B. xuefengmontis*, *B. yangmingensis* and *B. yingdeensis* (minimum SVL > 39.0 mm in the undescribed taxon vs. minimum SVL < 39.0 mm in the latter), and from *B. caudoprocta*, *B. fanjingmontis*, *B. jingdongensis*, *B. liboensis*, *B. mirabilis*, *B. omeimontis*, *B. qianbeiensis*, *B. sangzhiensis*, *B. shuichengensis* and *B. spinata* (maximum SVL < 44.0 mm in the undescribed taxon species vs. minimum SVL > 47.0 mm in the latter).

By a small horn-like tubercle present at the edge of each upper eyelid, the undescribed taxon differs from *B. acuta*, *B. caudoprocta*, *B. jinggangensis*, *B. liboensis*, *B. mirabilis*, and *B. palpebralespinosa* (vs. having a prominent and elongated tubercle in the latter).

By the vomerine ridges absent, the undescribed taxon differs from *B. acuta*, *B. angka*, *B. anlongensis*, *B. baishanzuensis*, *B. binlingensis*, *B. brachykolos*, *B. caudoprocta*, *B. cheni*, *B. daiyunensis*, *B. daweimontis*, *B. dongguanensis*, *B. elongata*, *B. fanjingmontis*, *B. fansipanensis*, *B. fengshunensis*, *B. frigida*, *B. hoanglienensis*, *B. insularis*, *B. jiangi*, *B. jingdongensis*, *B. jinggangensis*, *B. jiulianensis*, *B. kuatunensis*, *B. liboensis*, *B. lini*, *B. lushuiensis*, *B. mufumontana*, *B. nankunensis*, *B. nanlingensis*, *B. obesa*, *B. omeimontis*, *B. palpebralespinosa*, *B. pepe*, *B. puningensis*, *B. qianbeiensis*, *B. rubrimera*, *B. sangzhiensis*, *B. shimentaina*, *B. shuichengensis*, *B. spinata*, *B. tongboensis*, *B. wugongensis*, *B. xiangnanensis*, *B. xianjuensis*, *B. yangmingensis*, *B. yaoshanensis*, *B. yingdeensis* and *B. yunkaiensis* (vs. present in the latter).

By vomerine teeth absent, the undescribed taxon differs from *B. brachykolos*, *B. caudoprocta*, *B. daiyunensis*, *B. daweimontis*, *B. dongguanensis*, *B. elongata*, *B. fansipanensis*, *B. fengshunensis*, *B. frigida*, *B. hoanglienensis*, *B. insularis*, *B. jingdongensis*, *B. jinggangensis*, *B. jiulianensis*, *B. liboensis*, *B. nankunensis*, *B. nanlingensis*, *B. omeimontis*, *B. palpebralespinosa*, *B. puningensis*, *B. qianbeiensis*, *B. rubrimera*, *B. shimentaina*, *B. tongboensis*, and *B. yingdeensis* (vs. present in the latter).

By a small horn-like tubercle present at the edge of each upper eyelid, the undescribed taxon differs from *B. acuta*, *B. caudoprocta*, *B. jinggangensis*, *B. liboensis*, *B. mirabilis*, and *B. palpebralespinosa* (vs. having a prominent and elongated tubercle in the latter).

By tongue not notched behind, the undescribed taxon differs from *B. baolongensis*, *B. binlingensis*, *B. boettgeri*, *B. cheni*, *B. fanjingmontis*, *B. hoanglienensis*, *B. insularis*, *B. jingdongensis*, *B. jiulianensis*, *B. kuatunensis*, *B. liboensis*, *B. lushuiensis*, *B. minor*, *B. nanlingensis*, *B. omeimontis*, *B. pepe*, *B. qianbeiensis*, *B. sangzhiensis*, *B. sanmingensis*, *B. shuichengensis*, *B. spinata*, and *B. tongboensis* (vs. tongue feebly notched behind in the latter).

By toes without lateral fringes, the undescribed taxon differs from *B. binchuanensis*, *B. boettgeri*, *B. cheni*, *B. fanjingmontis*, *B. jingdongensis*, *B. liboensis*, *B. lini*, *B. palpebralespinosa*, *B. qianbeiensis*, *B. sanmingensis*, *B. shuichengensis*, *B. spinata*, and *B. xiangnanensis* (vs. having wide lateral fringes on toes in the latter).

By toes lacking webbing, the undescribed taxon differs from *B. jingdongensis*, *B. palpebralespinosa*, *B. qianbeiensis*, *B. shuichengensis*, *B. spinata*, and *B. wushanensis* (vs. toes with one-fourth in the latter).

By heels overlapping when thighs are positioned at right angles to the body, the undescribed taxon differs from *B. acuta*, *B. brachykolos*, *B. daoji*, *B. dongguanensis*, *B. fengshunensis*, *B. gaolanensis*, *B. hengshanensis*, *B. hungtai*, *B. insularis*, *B. kuatunensis*, *B. lichun*, *B. nankunensis*, *B. obesa*, *B. ombrophila*, *B. pepe*, *B. puningensis*, *B. shuichengensis*, and *B. wugongensis* (vs. not meeting in the latter) and differs from *B. binchuanensis*, *B. elongata*, *B. minor*, *B. xiangnanensis*, and *B. xuefengmontis* (vs. just meeting in the latter).

By tibiotarsal articulation reaching to the level to the middle of eye when leg stretched forward, the undescribed taxon differs from *B. cheni*, *B. congjiangensis*, *B. daweimontis*, *B. lini*, *B. obesa*, *B. shunhuangensis* and *B. yangmingensis* (vs. reaching the anterior corner of the eye or beyond eye or nostril and tip of snout in the latter); and differs from *B. brachykolos*, *B. hengshanensis*, *B. daoji*, *B. dongguanensis*, *B. dupanglingensis*, *B. fengshunensis*, *B. hungtai*, *B. insularis*, *B. jiangi*, *B. kuatunensis*, *B. lishuiensis*, *B. mufumontana*, *B. nankunensis*, *B. puningensis*, *B. qianbeiensis*, *B. shimentaina*, *B. xiangnanensis*, *B. xuefengmontis*, *B. yingdeensis* and *B. yunkaiensis* (vs. reaching the level between tympanum and eye or reach the tympanum or be even closer in the latter).

By having an internal single subgular vocal sac in males, the undescribed taxon differs from *B. caudoprocta* and *B. shuichengensis* (vs. vocal sac absent in the latter).

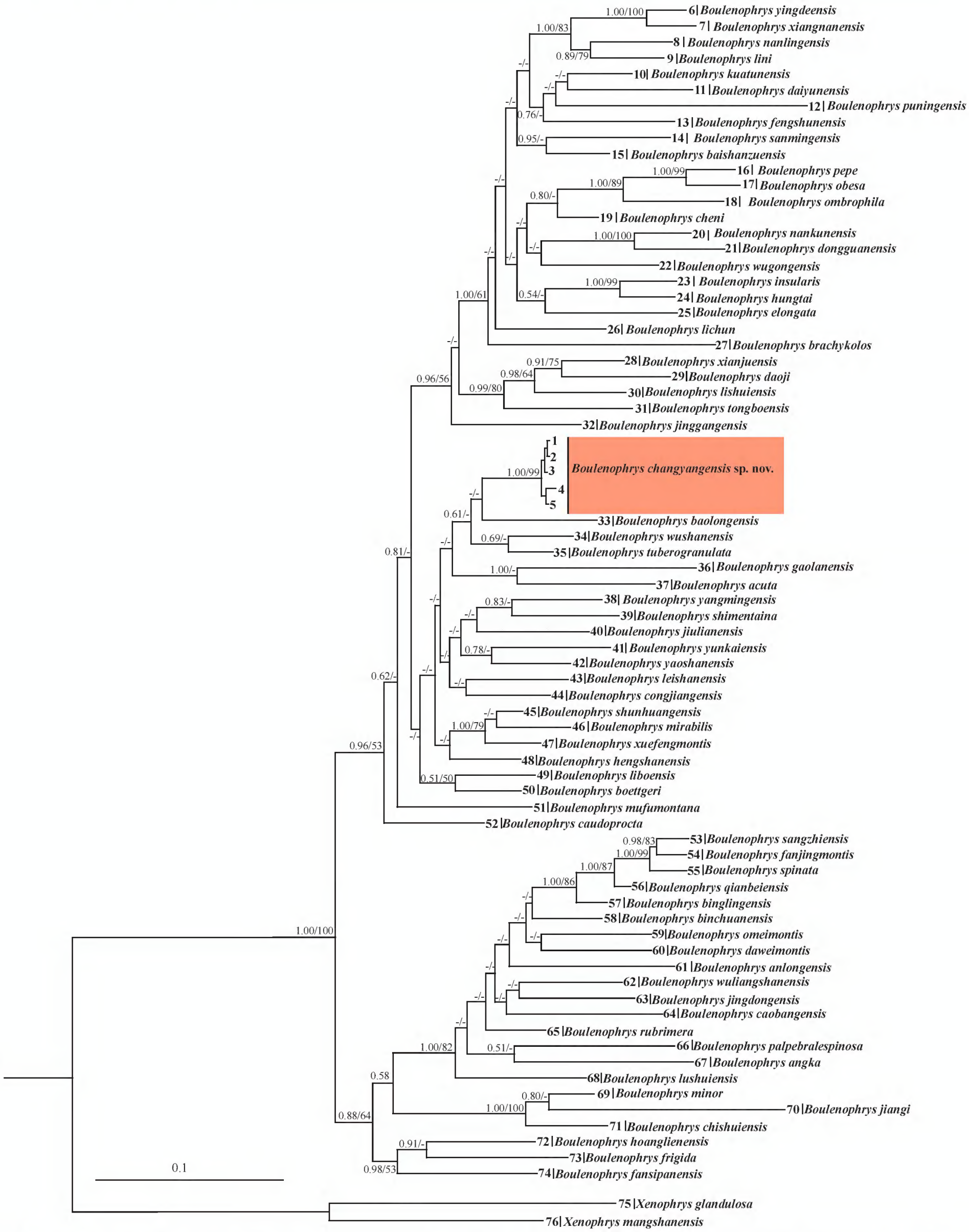


**Figure 2.** BI tree of the genus *Boulenophrys* reconstructed based on the 16S and COI gene sequences. Bayesian posterior probability/ML bootstrap supports were denoted beside each node, and "-" denotes BS < 50% or BPP < 0.60. Samples 1–76 refer to Table 1.

The undescribed taxon is phylogenetically most closely related to *B. baolongensis*, *B. wushanensis*, and *B. tuberogranulata* (Fig. 2). We examined specimens of *B. baolongensis* and *B. wushanensis* based on topotype specimens and of *B. tuberogranulata* based on its holotype (Fig. 3).

The undescribed taxon differs from *B. baolongensis* by the absence of large, pale-white circular spots on the shoulders (vs. present; Fig. 3B1); vomerine ridges weak and vomerine teeth absent (vs. both vomerine ridges and vomerine teeth absent); heels overlapping when thighs

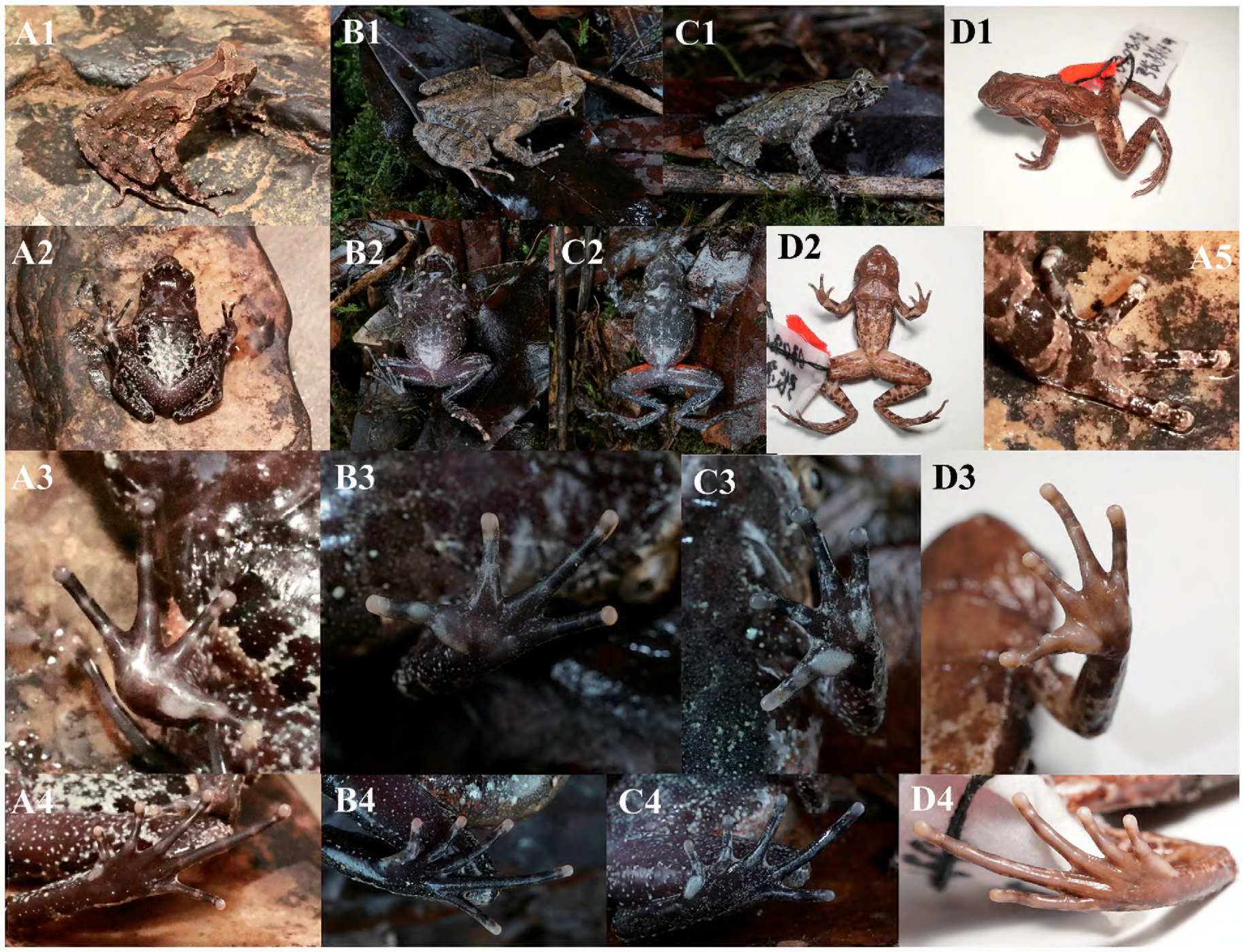


**Figure 3.** Photographs of *Boulenophrys changyangensis* sp. nov. and its closely related species. **A.** Holotype specimen MT CY20220806005 of *Boulenophrys changyangensis* sp. nov.; **B.** Topotype specimen WSA12 of *B. Baolongensis*; **C.** Topotype specimen WSA29 of *B. wushanensis*; **D.** Holotype specimen HUNL 03080902 of *B. tuberogranulata.* **A1–D1.** Dorsolateral view; **A2–D2.** Ventral view; **A3–D3.** Ventral view of hand; **A4–D4.** Ventral view of foot; **A5.** Dorsal view of hand.

are positioned at right angles to the body (vs. not meeting or just overlapping); head width larger than head length (vs. head width almost equal to head length).

The undescribed taxon differs from *B. wushanensis* by having a bigger body size in males (39.4–43.3 mm vs. 30.4–35.5 mm); vomerine ridges weak and vomerine teeth absent (vs. both vomerine ridges and vomerine teeth absent); lateral fringes absent in males (vs. lateral fringes present; Fig. 3C4); and heels overlapping when thighs are positioned at right angles to the body (vs. not meeting or just overlapping).

The undescribed taxon differs from *B. tuberogranulata* by head width larger than head length (vs. head width almost equal to head length); vomerine ridges weak (vs. well-developed); heels overlapping when thighs are positioned at right angles to the body (vs. not meeting or just meeting); and toes lacking webbing (vs. rudimentary; Fig. 3D4).

## Taxonomic accounts

Based on the results of these molecular phylogenetic and morphological comparisons, the specimens are distinct from all other congeners of Boulenophrys. The description of this new species is provided below.

***Boulenophrys changyangensis* sp. nov.**

https://zoobank.org/6F58A656-DC5A-4471-A522-9BEBCD3A92F9

**Type materials.** ***Holotype.*** • MT CY20220806005 (Figs 3, 4), adult male, from Changyang County, Hubei Province, CHINA (30.5732°N, 110.9404°E, ca. 616 m a. s. l.), collected by Jing Liu on 6 August 2022.

***Paratype.*** Four male specimens collected from the same place as holotype. • MT CY20220806002, MT CY20220806004, and MT CY20220806006 collected on 6 August 2022 by Shize Li.

**Diagnosis.** *Boulenophrys changyangensis* sp. nov. is assigned to the genus *Boulenophrys* based on molecular phylogenetic analyses and the following generic diagnostic characters: snout shield-like; projecting beyond the lower jaw; canthus rostralis distinct; chest glands small and round, closer to the axilla than to midventral line; femoral glands on rear part of thigh; vertical pupils (Fei and Ye 2016; Lyu et al. 2023).

*Boulenophrys changyangensis* sp. nov. could be distinguished from its congeners by a combination of the following morphological characters: body size moderate (SVL 39.4–43.3 mm in males); a small horn-like tubercle at edge of each upper eyelid; vomerine ridges and

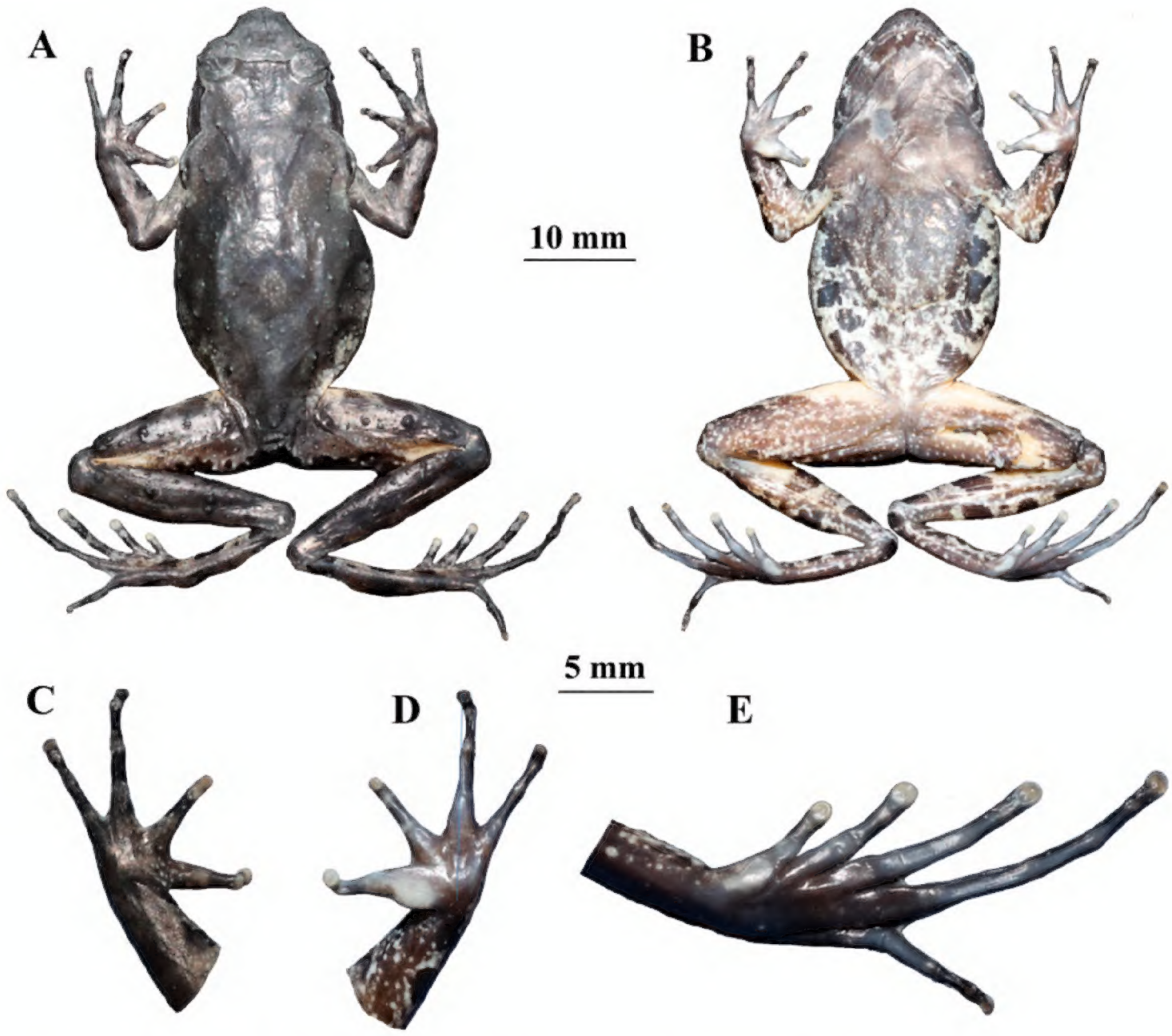


**Figure 4.** The holotype specimen MT CY20220806005 of *Boulenophrys changyangensis* sp. nov. (preserved) in **A.** Dorsal view; **B.** Ventral view; **C.** Dorsal view of hand; **D.** Ventral view of hand; **E.** Ventral view of foot.

vomerine teeth absent; tongue not notched behind; head width larger than head length; no large, pale-white circular spots on the shoulders; toes without webbing and lateral fringes; heels overlapped when thighs are positioned at right angles to the body; an internal single subgular vocal sac in males; in breeding males, the nuptial pads with black nuptial spines on the dorsal bases of the first and second fingers.

Description of holotype: MT CY20220806005 (Figs 3, 4). SVL 41.3 mm; other measurements are detailed in Table 3; head width larger than head length (HDW/HDL ratio about 1.12); snout rounded in dorsal view and protruding beyond lower jaw in profile; canthus rostralis distinct; loreal region sloping; dorsal surface of head flat; nostril orientated laterally, closer to snout than eye; internasal distance slightly larger than interorbital distance; eye large, eye diameter 35.1% of head length; tympanum distinct, upper margin in contact with supratympanic fold and TYP/EYE ratio 0.79; vomerine ridges and vomerine teeth absent; margin of tongue smooth, not notched behind; a single subgular vocal sac present.

Forelimbs slender, and the length of lower arm and hand is 48.0% of SVL; relative finger length I < II < IV < III; tips of fingers rounded, slightly dilated; fingers without webbing and lateral fringes; one subarticular tubercle present at base of each finger; two metacarpal tubercles, the inner one prominent and oblong, the outer one smaller and round.

Hindlimbs slender (HLL/SVL ratio 1.57); heels overlapping when thighs are positioned at right angles to the body, tibiotarsal articulation reaching the level to the middle of eye when leg stretched forward; tibia length longer than thigh length; relative toe lengths I < II < V < III < IV; tips of toes round, slightly dilated; subarticular tubercles present on the base of each toe; toes without webbing and lateral fringe; inner metatarsal tubercle is oval-shaped, and outer metatarsal tubercle is absent.

Dorsal skin rough; several tubercles on the flanks and dorsal surface of thighs and tibias; a small horn-like tubercle present at edge of upper eyelid; surface of upper eyelid rough, with small tubercles; supratympanic fold distinct, curved over tympanum; X-shaped skin ridge weak; two discontinuous dorsolateral parallel ridges on either side of the X-shaped ridges; Ventral surface with dense tubercles; small tubercles on surface of throat, chest, and forelimbs; glands on chest indistinct closer to axillae; femoral glands distinct, closer to the knee than cloaca.

**Coloration of holotype in life.** (Fig. 3). In life, dorsal surface brown; between the eyes, an inverted triangular dark-brown speckle is distinctly visible; the X-shaped ridges are dotted with dark-brown speckles; some dark-brown transverse bars on limbs and digits; loreal and temporal regions with brown mottling, iris light brown. The ventral surface of throat dark purple, cheat dark brown, belly

**Table 3.** Measurements of the five adult male specimens of *Boulenophrys changyangensis* sp. nov. Units are given in mm. See abbreviations for the morphological characters in the Materials and methods section.

| | MT CY20220806006 | MT CY20220806002 | MT CY20220806003 | MT CY20220806004 | MT CY20220806005 | Rang (mean ± SD ) |
|---|---|---|---|---|---|---|
| SVL | 39.4 | 43.3 | 41.5 | 42.0 | 41.3 | 39.4–43.3 (41.5 ± 1.4) |
| HDL | 10.9 | 11.4 | 12.3 | 11.4 | 11.9 | 10.9–12.3 (11.6 ± 0.6) |
| HDW | 12.9 | 12.7 | 13.1 | 12.9 | 13.6 | 12.7–13.6 (13.0 ± 0.3) |
| SL | 4.6 | 4.6 | 6.0 | 5.2 | 5.6 | 4.6–4.0 (5.2 ± 0.6) |
| IND | 5.1 | 4.4 | 5.3 | 4.8 | 4.5 | 4.4–5.3 (4.8 ± 0.4) |
| IOD | 4.1 | 4.0 | 4.2 | 4.3 | 4.6 | 4.0–4.6 (4.2 ± 0.2) |
| UEW | 3.1 | 3.7 | 3.4 | 3.8 | 3.7 | 3.1–3.8 (3.6 ± 0.3) |
| ED | 4.6 | 4.0 | 3.6 | 4.4 | 4.2 | 3.6–4.6 (4.2 ± 0.4) |
| TYD | 3.0 | 2.6 | 3.0 | 3.1 | 3.3 | 2.6–3.3 (3.0 ± 0.3) |
| LAL | 20.1 | 19.2 | 20.9 | 19.7 | 19.8 | 19.2–20.9 (19.9 ± 0.6) |
| LW | 2.6 | 3.0 | 2.8 | 3.0 | 3.0 | 2.6–3.0 (2.9 ± 0.2) |
| ML | 11.0 | 10.4 | 10.5 | 10.8 | 10.9 | 10.4–11.0 (10.7 ± 0.3) |
| HLL | 62.7 | 62.7 | 63.6 | 58.2 | 64.7 | 58.2–64.7 (62.4 ± 2.5) |
| THL | 17.9 | 17.6 | 18.5 | 15.9 | 17.5 | 15.9–18.5 (17.5 ± 1.0) |
| TL | 19.9 | 19.7 | 20.2 | 18.4 | 20.4 | 18.4–20.4 (19.7 ± 0.8) |
| TW | 3.5 | 4.0 | 4.5 | 4.5 | 4.1 | 3.5–4.5 (4.1 ± 0.4) |
| TFL | 29.8 | 28.4 | 28.7 | 27.2 | 30.0 | 27.2–30.0 (28.8 ± 1.1) |
| FL | 18.4 | 17.6 | 17.5 | 16.7 | 18.7 | 16.7–18.7 (17.8 ± 0.8) |

**Figure 5.** Color variation in *Boulenophrys changyangensis* sp. nov. **A.** Dorsolateral view of the specimen MT CY20220806003; **B.** Dorsolateral view of the specimen MT CY20220806006; **C.** Dorsolateral view and **D.** Ventral view of male specimen MT CY20220806004.

white with brown spots interspersed, and irregular black spots on both sides of the belly; surface of ventral limbs purple-brown, with white mottling; digits, inner metacarpal tubercles, and inner metatarsal tubercle grayish white; pectoral glands and femoral glands white (Fig. 4).

**Coloration of holotype in preservation.** (Fig. 4). Color of dorsal surface fades to brownness; the inverted triangular brown speckle between the eyes and X-shaped ridges on dorsum fades indistinct; the throat and anterior chest fade to light brownish, while the abdomen grayish white, fingers and toes grayish white, and the palms and soles dark purple with a reddish tinge (Fig. 4).

**Variation.** Measurement data for the type series are provided in Table 2. Morphological diagnostic characters observed in all paratypes were consistent with those of the holotype. However, there were variations in coloration and stripe patterns between individuals (Fig. 5). In MT CY20220806005, a distinct beige line between the inverted triangular markings and the X-shaped markings on dorsum; in MT CY20220806005, the

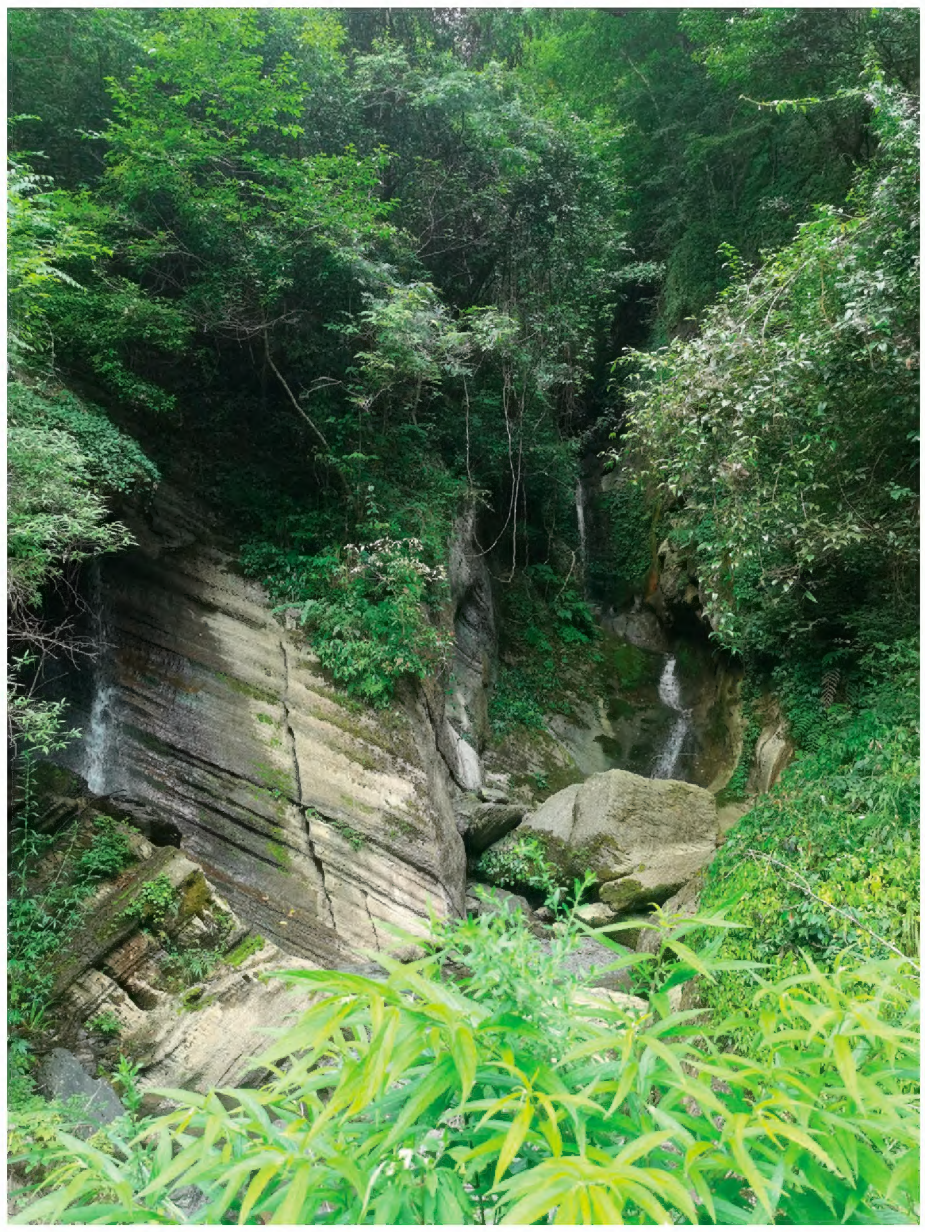

**Figure 6.** Habitats of *Boulenophrys changyangensis* sp. nov. at the type locality, Changyang County, Hubei Province, China.s

markings on dorsum in a net-like pattern, and the colors of the limbs and the posterior part of the trunk are darker; in MT CY20220806004, dorsal surface brown, scattered with small orange-red tubercles, and the abdomen smooth, mostly white with black patches interspersed (Fig. 5).

**Secondary sexual characters.** Adult males possess a single subgular vocal sac. During the breeding season, there are dark brown nuptial pads at the bases of the first and second fingers, and these pads are covered with fine, dense black nuptial spines.

**Distribution and habitats.** *Boulenophrys changyangensis* sp. nov. is known from the type locality, Changyang County, Hubei Province, China, at elevations of about 600 m. The individuals of the new species were frequently found on the stones in the streams surrounded by evergreen broadleaved forests (Fig. 6), and only one sympatric amphibian species, *Nanorana quadranus* (Liu, Hu, & Yang, 1960), was found.

**Etymology.** The specific epithet changyang refers to the distribution of the species, Changyang County, Hubei Province, China. The suggested common English name is "Changyang Horned Toad," and the Chinese name is "Cháng Yáng Jiǎo Chán (长阳角蟾)" for this species.

## Discussion

Currently, a total of 71 species of the genus *Boulenophrys* have been recorded globally, among which 66 species are distributed in China (Frost 2025). It is worth noting that approximately 66% of the species (47 species) were described in the past decade. Previous studies have shown that species of the genus *Boulenophrys* exhibit weak dispersal ability and occupy narrow ecological niches, similar to other cryptic species (Cheng et al. 2025), which has led to the restricted distribution ranges of most species (Wang et al. 2019a; Lyu et al. 2023). In this study, we describe a new species of the genus *Boulenophrys* collected from Changyang County, Hubei Province. Although the phylogenetic relationship between *Boulenophrys changyangensis* sp. nov. and its congeners remains unresolved (Fig. 2), this may be due to the limited phylogenetic resolution provided by the short length of the selected gene fragments (Chan et al. 2022). Nevertheless, the morphology of the new species shows clear distinctions from related species. Diagnostic characteristics include the absence of large pale-white circular spots on the shoulders, medium body size, lack of vomerine ridges, absence of toe webbing, and absence of lateral toe fringes. These features effectively distinguish it from its congeners. Furthermore, the straight-line distances between the type locality of *Boulenophrys changyangensis* sp. nov. and those of *B. baolongensis*, *B. wushanensis*, and *B. tuberogranulata* all exceed 100 kilometers. We therefore speculate that geographic isolation likely promoted speciation between this lineage and its relatives, driving the evolution of distinct morphologies in the new species.

Changyang lies within the northeastern extension of the Wuling Mountains, a region characterized by complex geological history and well-preserved vegetation. In recent years, new amphibian species have been frequently reported in the Guizhou and Hunan sections of the Wuling Mountains. Moreover, existing research has shown that such geographically isolated environments can promote species differentiation by limiting gene flow (Jin et al. 2022; Zhao et al. 2022). Therefore, it remains unclear whether additional cryptic amphibian species exist in this region. However, most surveys to date have been concentrated in the Guizhou and Hunan sections of the Wuling Mountains, with a serious lack of systematic research in the Hubei section. We therefore recommend prioritizing interdisciplinary research in these under-investigated areas to comprehensively assess cryptic diversity.

## Acknowledgements

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# Supplementary material 1

## Uncorrected *p*-distances between the *Boulenophrys* species based on the 16S gene sequences

Authors: Shize Li, Shengchao Shi, Jing Liu, Jingjing Zhao, Shuo Gao, Bin Wang

Data type: xlsx

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Link: https://doi.org/10.3897/zse.101.155859.suppl1

# Supplementary material 2

## Uncorrected *p*-distances between the *Boulenophrys* species based on the COI gene sequences

Authors: Shize Li, Shengchao Shi, Jing Liu, Jingjing Zhao, Shuo Gao, Bin Wang

Data type: xlsx

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# Supplementary material 3

## Diagnostic characters separating *Boulenophrys changyangensis* sp. nov. from other species of *Boulenophrys*

Authors: Shize Li, Shengchao Shi, Jing Liu, Jingjing Zhao, Shuo Gao, Bin Wang

Data type: xlsx

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